CompCytogen 5(5): 397–410 (2011)
doi: 10.3897/CompCytogen.v5i5.1589
www.pensoft.net/journals/compcytogen

RESEARCH ARTICLE

COMPARATIVE Cytogenetics
A peer-reviewed open-access journal
International Journal of Plant & Animal Cytogenetic, Karyosystematics, and Molecular Systematics

# Cytogenetic characterization of four species of the genus *Hypostomus* Lacépède, 1803 (Siluriformes, Loricariidae) with comments on its chromosomal diversity

Marceléia Rubert[1], Renata da Rosa[2], Fernando Camargo Jerep[3], Luiz Antônio Carlos Bertollo[1], Lucia Giuliano-Caetano[2]

**1** *Departamento de Genética e Evolução, Universidade Federal de São Carlos, Rodovia Washington Luís, km 235 - SP-310, P.O. Box 676, CEP 13565-905, São Carlos, São Paulo, Brazil* **2** *Departamento de Biologia Geral, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, Pr 445 Km 380, Campus Universitário, P.O. Box 6001, CEP 86051-970, Londrina, Paraná, Brazil* **3** *Museu de Ciências e Tecnologia, Pontifícia Universidade Católica, Av. Ipiranga, 6681, P.O. Box 1429, CEP 90619-900, Porto Alegre, Rio Grande do Sul, Brazil*

Corresponding author: *Marceléia Rubert* (marcerubert@hotmail.com)

Academic editor: *G. Furgala-Selezniow* | Received 21 April 2011 | Accepted 8 August 2011 | Published 22 December 2011

**Citation:** Rubert M, da Rosa R, Jerep FC, Bertollo LAC, Giuliano-Caetano L (2011) Cytogenetic characterization of four species of the genus *Hypostomus* Lacépède, 1803 (Siluriformes, Loricariidae) with comments on its chromosomal diversity. Comparative Cytogenetics 5(5): 397–410. doi: 10.3897/CompCytogen.v5i5.1589

## Abstract

Cytogenetic analyses were performed on fishes of the genus *Hypostomus* (*H. ancistroides* (Ihering, 1911), *H. strigaticeps* (Regan, 1908), *H. regani* (Ihering, 1905), and *H. paulinus* (Ihering, 1905)) from the seven tributaries of the Paranapanema River Basin (Brazil) by means of different staining techniques (C-, Ag-, $CMA_3$- and DAPI-banding) and fluorescence in situ hybridization (FISH) to detect 18S rDNA sites. All species showed different diploid numbers: 2n=68 (10m+26sm+32st-a) in *H. ancistroides*, 2n=72 (10m+16sm+46st-a) in *H. strigaticeps*, 2n=72 (10m+18sm+44st-a) in *H. regani* and 2n=76 (6m+16sm+54st-a) in *H. paulinus*. Ag-staining and FISH revealed various numbers and locations of NORs in the group. NORs were usually located terminally on the subtelocentric/acrocentric chromosomes: on the long arm in *H. strigaticeps* (2 to 4) and *H. paulinus* (2); and on the short arm in *H. ancistroides* (2 to 8) and *H. regani* (2 to 4). Conspicuous differences in heterochromatin distribution and composition were found among the species, terminally located in some st-a chromosomes in *H. ancistroides*, *H. strigaticeps*, and *H. paulinus*, and interstitially dispersed in most st-a chromosomes, in *H. regani*. The fluorochrome staining indicated that different classes of GC and/or AT-rich repetitive DNA evolved in this group. Our results indicate that chromosomal rearrangements and heterochromatin base-pair composition were significant events during the course of differentiation of this group. These features emerge as an excellent cytotaxonomic marker, providing a better understanding of the evolutionary mechanisms underlying the chromosomal diversity in *Hypostomus* species.

Copyright *Marceléia Rubert et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License 3.0 (CC-BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Keywords**
loricariid catfishes, chromosome banding, NORs, fluorochromes, fluorescence in situ hybridization (FISH)

## Introduction

The suckermouth armored catfishes *Hypostomus* Lacépède, 1803 (Siluriformes, Loricariidae) represent one of the most specious genus of the family Loricariidae, with 127 nominal species (Zawadzki et al. 2008).

Most species of this family have a wide distribution in Central and South America. They usually dwell in the rapids, but may be present in different aquatic habitats and in sand banks or rocky rivers. The species of Hypostominae are restricted to freshwater habitats, with the exception of *Hypostomus watwata* Hancock, 1828, which is a benthic species that lives in estuarine waters. Most of these animals have twilight habits and during daylight hours remain under stones or trunks of dead trees (Weber 2003).

The taxonomy of the Loricariidae family has constantly been reviewed through morphological studies (Reis et al. 2006), molecular phylogenies (Montoya-Burgos et al. 1998), allozymes (Zawadzki et al. 2005), and cytogenetic studies (Artoni and Bertollo 2001, Alves et al. 2006). In the most recent taxonomic study (Reis et al. 2006), this family was subdivided into six subfamilies: Lithogeneinae, Neoplecostominae, Hypoptopomatinae, Loricariinae, Hypostominae, and a new subfamily, Delturinae.

Among Hypostominae, only eight of its 30 genera (Armbruster 2004), namely *Ancistrus* Kner, 1854, *Hemiancistrus* Bleeker, 1862, *Hypostomus*, *Baryancistrus* Rapp Py-Daniel, 1989, *Panaque* Eigenmann and Eigenmann, 1889, *Pogonopoma* Regan, 1904, *Pterygoplichthys* Gill, 1858, and *Rhinelepis* Agassiz, 1829, have been object of cytogenetic studies. However, most of these reports are limited to the diploid number, silver staining of the nucleolus organizer regions (Ag-NORs), and chromosome C-banding (Artoni and Bertollo 2001, Alves et al. 2006). Among these genera, *Hypostomus* has the largest number of karyotyped species; however, the number of the studied species versus the species ascribed to the genus is scarce, i.e. approximately 10% (Table 1).

Concerning the cytotaxonomy, this genus shows a wide variation in diploid number, ranging from 2n=52 in *H. emarginatus* Valenciennes, 1840 (Artoni and Bertollo 2001) to 2n=84 in *Hypostomus* sp. 2-Rio Perdido NUP 4249 (Cereali et al. 2008). The most frequent diploid number was 2n=72 (Table 1). The occurrence of multiple NORs located in terminal position on the chromosomes is most common in this genus (Artoni and Bertollo 2001). Regarding the repetitive DNA in *Hypostomus*, different classes of GC and/or AT-rich heterochromatin, usually with segments located in terminal and/or interstitial chromosome regions, were observed in this fish group (Artoni and Bertollo 1999, Kavalco et al. 2004, Cereali et al. 2008).

**Table 1.** A summary of cytogenetic data available for the genus *Hypostomus*.

| Species | Locality | 2n | FN | KF | NORs | CB | Ref. |
|---|---|---|---|---|---|---|---|
| *Hypostomus affinis* (Steindachner, 1877) | Jacuí stream (SP) | 66 | 94 | 14m 14sm 12st 26a | 5,t, la | t, la,pc | 9,10 |
| *Hypostomus albopunctatus* (Regan, 1908) | Mogi-Guaçu river (SP) | 74 | 104 | 10m 20sm 44st-a | 6,t,sa,la | n.d. | 3 |
| *Hypostomus albopunctatus* | Piracicaba river (SP) | 74 | 104 | 10m 20sm 44st-a | 3,t,sa,la | i,la,t,sa,pc | 7 |
| *Hypostomus ancistroides* | n.d. | 68 | 106 | 10m 28sm 30st-a | n.d. | n.d. | 2 |
| *Hypostomus ancistroides* | Mogi-Guaçu river (SP) | 68 | 102 | 16m 18sm 34st-a | 6,t,sa | n.d. | 3 |
| *Hypostomus ancistroides* | Araquá river (SP) | 68 | 96 | 18m 10sm 12st 28a | 6,t,sa | n.d. | 12 |
| *Hypostomus ancistroides* | *** | 68 | 104 | 10m 26sm 32st-a | 6,t,sa | t,la,pc | 16 |
| *Hypostomus* prope *auroguttatus* Kner, 1854 | Mogi-Guaçu river (SP) | 76 | 114 | 8m 30sm 38st-a | 2,t,la | n.d. | 3 |
| *Hypostomus cochliodon* Kner, 1854 | Salobra river and Salobrinha stream (MS) | 64♂ | 100 | 16m 20sm 28st-a | n.d. | t,la | 11 |
| | | 64♀ | 97 | 16m 19sm 27st-a | n.d. | t,la | 11 |
| *Hypostomus emarginatus* | Araguaia river (MT) | 52 | 98 | 16m 30sm 6st | 2,t,la | n.d. | 5 |
| *Hypostomus goyazensis* (Regan, 1908) | Vermelho river (GO) | 72 | 98 | 10m 16sm 10st 36a | 2,t,sa | n.d. | 12 |
| *Hypostomus macrops* (Eigenmann et Eigenmann, 1888) | n.d. | 68 | 92 | 10m 14sm 44st-a | n.d. | n.d. | 2 |
| *Hypostomus nigromaculatus* (Schubart, 1964) | Mogi-Guaçu river (SP) | 76 | 104 | 8m 20sm 48st-a | 3,t,la | t,la,pc | 15 |
| *Hypostomus nigromaculatus* | Três Bocas stream (PR) | 76 | 102 | 6m 20sm 50st-a | 3,t,sa,la | t,la,sa,pc | 15 |
| *Hypostomus paulinus* | n.d. | 74 | 104 | 10m 20sm 44st-a | n.d. | n.d. | 2 |
| *Hypostomus paulinus* | Três Bocas and Apertados streams (PR) | 76 | 98 | 6m 16sm 54st-a | 2,t,la | t,la,pc | 16 |
| *Hypostomus plecostomus* (Linnaeus, 1758) | | 54 | 90 | 24m 12sm 18st-a | n.d. | n.d. | 1 |
| *Hypostomus regani* | Mogi-Guaçu river (SP) | 72 | 102 | 10m 20sm 42st-a | n.d. | n.d. | 3 |
| *Hypostomus regani* | Araquá river (SP) | 72 | 102 | 12m 18sm 26st 16a | 4,t,la | n.d. | 12 |
| *Hypostomus regani* | Piumhi river (MG) | 72 | 116 | 8m 16sm 48st-a | 4,t,la | i | 13 |
| *Hypostomus regani* | Jacutinga river | 72 | 100 | 10m 18sm 44st-a | 4,t,sa | i,pc | 16 |
| *Hypostomus strigaticeps* | n.d. | 74 | 86 | 8m 4sm 62st-a | n.d. | n.d. | 2 |

| Species | Locality | 2n | FN | KF | NORs | CB | Ref. |
|---|---|---|---|---|---|---|---|
| *Hypostomus strigaticeps* | *** | 72 | 98 | 10m 16sm 46st-a | 4,t,la | t,la,pc | 16 |
| *Hypostomus* sp. A | Córrego Rincão (SP) | 70 | 102 | 18m 14sm 38st-a | 4,t,sa,la | n.d. | 3 |
| *Hypostomus* sp. B | Mogi-Guaçu river (SP) | 72 | 102 | 12m 18sm 42st-a | 2,t,la | t,la,pc | 3,4 |
| *Hypostomus* sp. C | Mogi-Guaçu river (SP) | 72 | 102 | 10m 18sm 44st-a | 4,t,la | n.d. | 3 |
| *Hypostomus* sp. D1 | Mogi-Guaçu river (SP) | 72 | 108 | 10m 26sm 36st-a | 4,t,la | n.d. | 3 |
| *Hypostomus* sp. D2 | Mogi-Guaçu river (SP) | 72 | 106 | 14m 20sm 38st-a | 4,t,la | n.d. | 3 |
| *Hypostomus* sp. E | Mogi-Guaçu river (SP) | 80 | 104 | 8m 16sm 56st-a | 2,t,sa | t,la,sa,i,pc | 3,4 |
| *Hypostomus* sp. F | São Francisco river (MG) | 76 | 102 | 10m 16sm 50st-a | n.d. | pc,t,i | 4 |
| *Hypostomus* sp. G | Araguaia river (MT) | 64 | 102♂ | 14m 24sm 26st-a | 2,sa | pc,t,i | 6 |
| | | 64 | 103♀ | 15m 24sm 25st-a | 2,sa | pc,t,i | 6 |
| *Hypostomus* sp.1 | Paranapanema river (SP) | 64 | n.d. | n.d. | n.d. | n.d. | 8 |
| *Hypostomus* sp.2 | Alambari and Jacutinga streams (SP) | 68 | n.d. | n.d. | n.d. | n.d. | 8 |
| *Hypostomus* sp. 3 | Quinta and Edgardia stream, Paranapanema river (SP) | 72 | n.d. | n.d. | n.d. | n.d. | 8 |
| *Hypostomus* sp. 4 | Paranapanema river; Hortelã stream (SP) | 76 | n.d. | n.d. | n.d. | n.d. | 8 |
| *Hypostomus* sp. 2-rio Perdido NUP 4249 | Perdido river (MS) | 84 | 106 | 6m 16sm 62st-a | 2,t,la | pc,t,la | 14 |
| *Hypostomus* sp. 3-córrego Salobrinha NUP 4247 | Salobra river and Salobrinha stream (MS) | 82 | 102 | 6m 14sm 62st-a | 2,t,la | pc,t,la | 14 |
| *Hypostomus* sp.1a | Patos stream (MG) | 76 | 106 | 6m 8sm 62st-a | 3,t,sa,la | t,la | 13 |
| *Hypostomus* sp.1b | Araras stream (MG) | 76 | 106 | 6m 8sm 62st-a | 3,sa,la | t,la | 13 |
| *Hypostomus* sp.2 | Araras stream (MG) | 74 | 106 | 10m 6sm 58st-a | 2,la | t,la | 13 |

Diploid numbers (2n), number fundamental (NF), karyotype formula (KF), metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a); *** several collection sites of the Paranapanema river basin. Number of nucleolar organizing region (NORs), C-banding (CB). Interstitial (i), terminal (t), pericentromeric (pc), short arm (sa), long arm (la). No data (n.d.). References (Ref.): (1) Muramoto et al. (1968), (2) Michele et al. (1977), (3) Artoni and Bertollo (1996), (4) Artoni and Bertollo (1999), (5) Artoni and Bertollo (2001), (6) Artoni et al. (1998), (7) Camilo (2004), (8) Fenerich et al. (2004), (9) Kavalco et al. (2004), (10) Kavalco et al. (2005), (11) Cereali (2006), (12) Alves et al. (2006), (13) Mendes Neto (2008), (14) Cereali et al. (2008), (15) Rubert et al. (2008), (16) Present study.

The aim of this work was to analyze specimens of four species of the genus *Hypostomus* from different populations of the Paranapanema River Basin by means of conventional and molecular cytogenetic techniques and compare the obtained data with the cytogenetic records available for other species of the genus.

## Material and methods

Cytogenetic analysis was performed on a total of 148 specimens of four *Hypostomus* species collected at different sites of the Paranapanema River Basin (southern Brazil) (Table 1). The specimens were deposited in the Museu de Zoologia of the Universidade Estadual de Londrina (MZUEL), Londrina, Paraná State, Brazil.

***Conventional staining.*** Metaphase chromosomes were obtained through the air-drying technique (Bertollo et al. 1978) and stained with 5% Giemsa stain solution (diluted with phosphate buffer, pH 6.8). The karyotypes were organized in groups of metacentric (m), submetacentric (sm), and subtelocentric-acrocentric (st-a) chromosomes.

***Chromosome banding.*** C-banding was performed according to Sumner (1972). The silver staining of the nucleolus organizer regions (Ag-NORs) was performed according to Howell and Black (1980). The GC- and AT-rich bands were detected by staining with Chromomycin $A_3$ ($CMA_3$) and 4'6-diamidin-2-phenylindole (DAPI), respectively, according to Schweizer (1980). The slides were stained with 0.5 mg/mL $CMA_3$ for 1 h, washed in distilled water and sequentially stained with 2 μg/mL DAPI for 15 min. Slides were mounted with a medium composed of glycerol/McIlvaine buffer (pH 7.0) 1:1 supplemented with 2.5 mM $MgCl_2$.

***Fluorescence in situ hybridization (FISH).*** The fluorescence in situ hybridization procedure was performed according to Swarça et al. (2001). The 18S rDNA probe of *Prochilodus argenteus* Spix and Agassiz, 1829 (Hatanaka and Galetti Jr 2004) was labeled with biotin-14-dATP by nick translation. Slides were treated with 30 μL of the hybridization mixture containing 100 ng of labeled probe (4 μL), 50% formamide (15 μL), 50% polyethylene glycol (6 μL), 20xSSC (3 μL), 100 ng of calf thymus DNA (1 μL) and 10% SDS (1 μL). The slides and the hybridization mixture were denatured at 90°C for 30 min in a Termocycler, and hybridization was performed overnight at 37°C in a humidified chamber. Post-hybridization washes were carried out in 2x SSC, 20% formamide in 0.1x SSC and 4xSSC/0.2% Tween 20, all at 42°C. The hybridized probe was detected with FITC-conjugated avidin. The post-detection washes were performed in 4xSSC/0.2% Tween 20 at RT. The slides were mounted in 23 μL DABCO solution consisting of the following: 90% glycerol, 2% Tris HCl 20 mM, pH 8.0, and 2.3% (wt/vol) 1,4-diazabicyclo (2,2,2) octane, pH 8.6), 1 μL of propidium iodate (1 μg/mL) and 1 μL of $MgCl_2$ 50 mM.

Images were acquired with Leica DM 4500 B microscope equipped with a DFC 300FX camera and Leica IM50 4.0 software.

## Results

Specimens of *Hypostomus ancistroides* showed a diploid number 2n=68 and a fundamental number (FN) of 104, with a karyotype formula of 10m+26sm+32st-a. One chromosome of pair 26 showed size heteromorphism (Fig. 1a). Silver nitrate staining (Fig. 1a left box) and FISH (Fig. 1a right box) revealed up to four pairs of subtelocentric/acrocentric NOR-bearing chromosomes. $CMA_3$ marked the terminal region of the long arms of pair 26, the pericentromeric region of the second pair of metacentric chromosomes, and probably the NOR-bearing chromosomes (Fig. 2a). No fluorescent staining was observed after DAPI staining (Fig. 2b). Heterochromatin was distributed in the pericentromeric region of the second pair (m) of the complement and in the terminal region of the long arm (pair 26) (Fig. 3a).

*Hypostomus strigaticeps* presented a diploid number 2n=72 and a FN of 98, with a karyotype formula of 10m+16sm+46st-a (Fig. 1b). The Ag-NOR site numbers ranged from two to four marked chromosomes (st-a) located in the terminal region of the long arm (pairs 18 and 28) (Fig. 1b left box), similar to the number observed in FISH (Fig. 1b right box). $CMA_3$ marked four chromosomes, possibly the Ag-NOR sites, and the pericentromeric regions of most subtelocentric/acrocentric chromosomes (Fig. 2c). Staining with DAPI revealed large blocks in the terminal regions of four-eight subtelocentric/acrocentric chromosomes (Fig. 2d). C-banding revealed the occurrence of heterochromatic blocks in the pericentromeric region of the third pair of metacentric chromosomes and of up to eight large blocks in the terminal regions of the long arms of subtelocentric/acrocentric chromosomes. In one of those chromosome pairs, the heterochromatic block was adjacent to the secondary constriction (Fig. 3b).

*Hypostomus regani* had 2n=72 with a karyotype formula of 10m+18sm+44st-a and FN of 100 (Fig. 1c). Ag-NORs were located in the terminal position on the short arms of four subtelocentric/acrocentric chromosomes (pairs 26 and 27) (Fig. 1c left box). The same number of NOR-bearing chromosomes was observed after FISH (Fig. 1c right box) and $CMA_3$-staining (Fig. 2e). Interstitial $CMA_3$-negative blocks were observed in most of the subtelocentric/acrocentric chromosomes, which, in contrast, were positive after DAPI staining (Fig. 2f). Heterochromatin was distributed in the interstitial region of most st-a chromosomes and in the pericentromeric region of one metacentric pair (Fig. 3c).

*Hypostomus paulinus* showed 2n=76, FN=98 and a karyotype formula of 6m+16sm+54st-a (Fig. 1d). NORs were located in the terminal position on the long arms of chromosome pair 16 (Fig. 1d left box), similar to the chromosomes observed in FISH (Fig. 1d right box). $CMA_3$-banding marked up to eight chromosomes (st-a) with large GC-rich blocks, and one st-a pair, probably corresponding to NOR-bearing chromosomes, and in the pericentromeric region of the first (m) pair (Fig. 2g); after DAPI staining, eight fluorescent bands were observed (Fig. 2h). Heterochromatin was distributed in the pericentromeric region of the first pair of metacentric chromosomes, in the terminal region of the long arms of eight pairs of

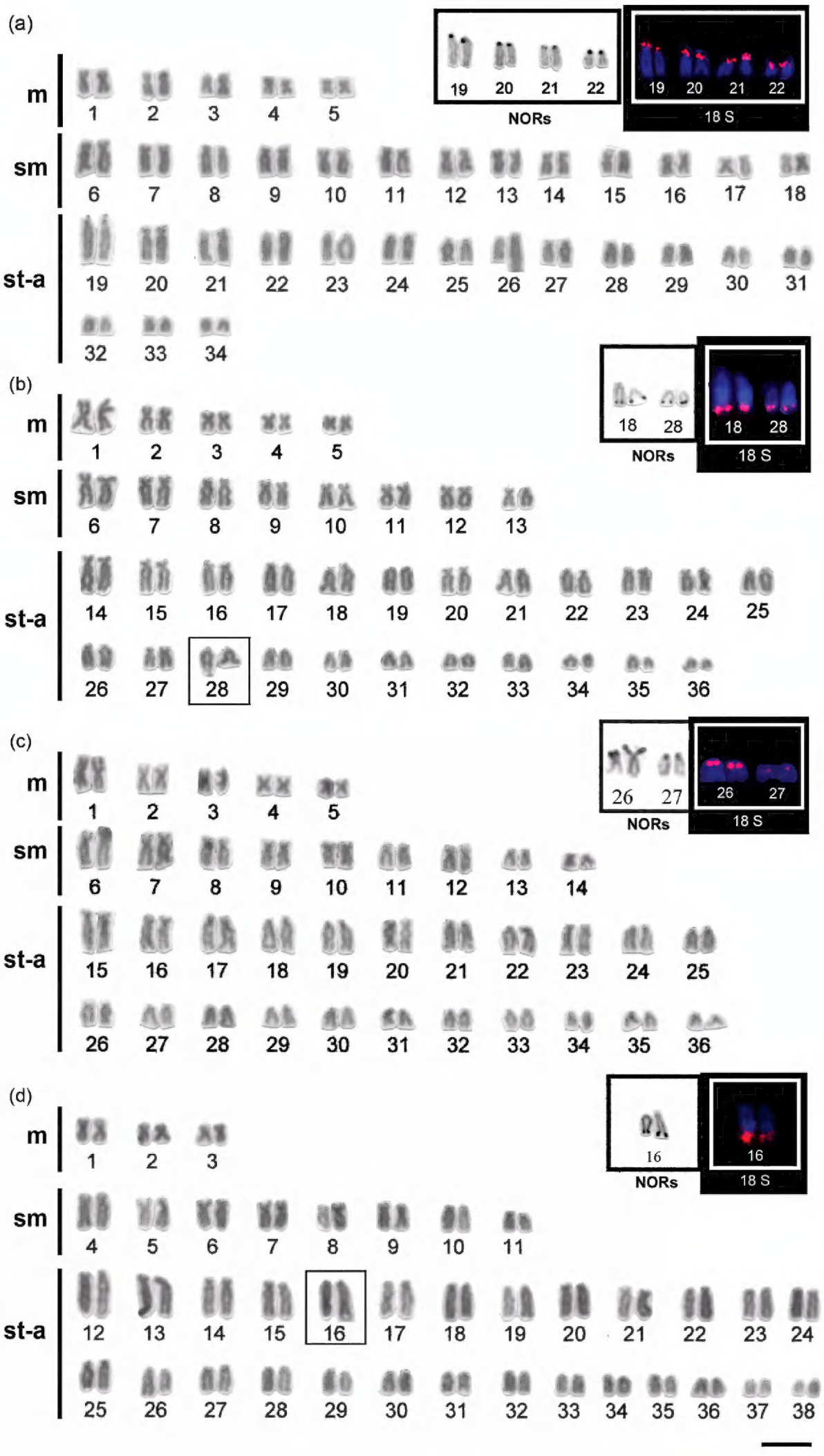


**Figure 1.** Karyotypes of **a** *H. ancistroides* **b** *H. strigaticeps* **c** *H. regani* **d** *H. paulinus* arranged from Giemsa-stained chromosomes. In the insets, partial karyotypes of the NOR-bearing chromosome pairs after Ag-staining (left) and FISH with 18S rDNA probe (right). Bar = 10 μm.

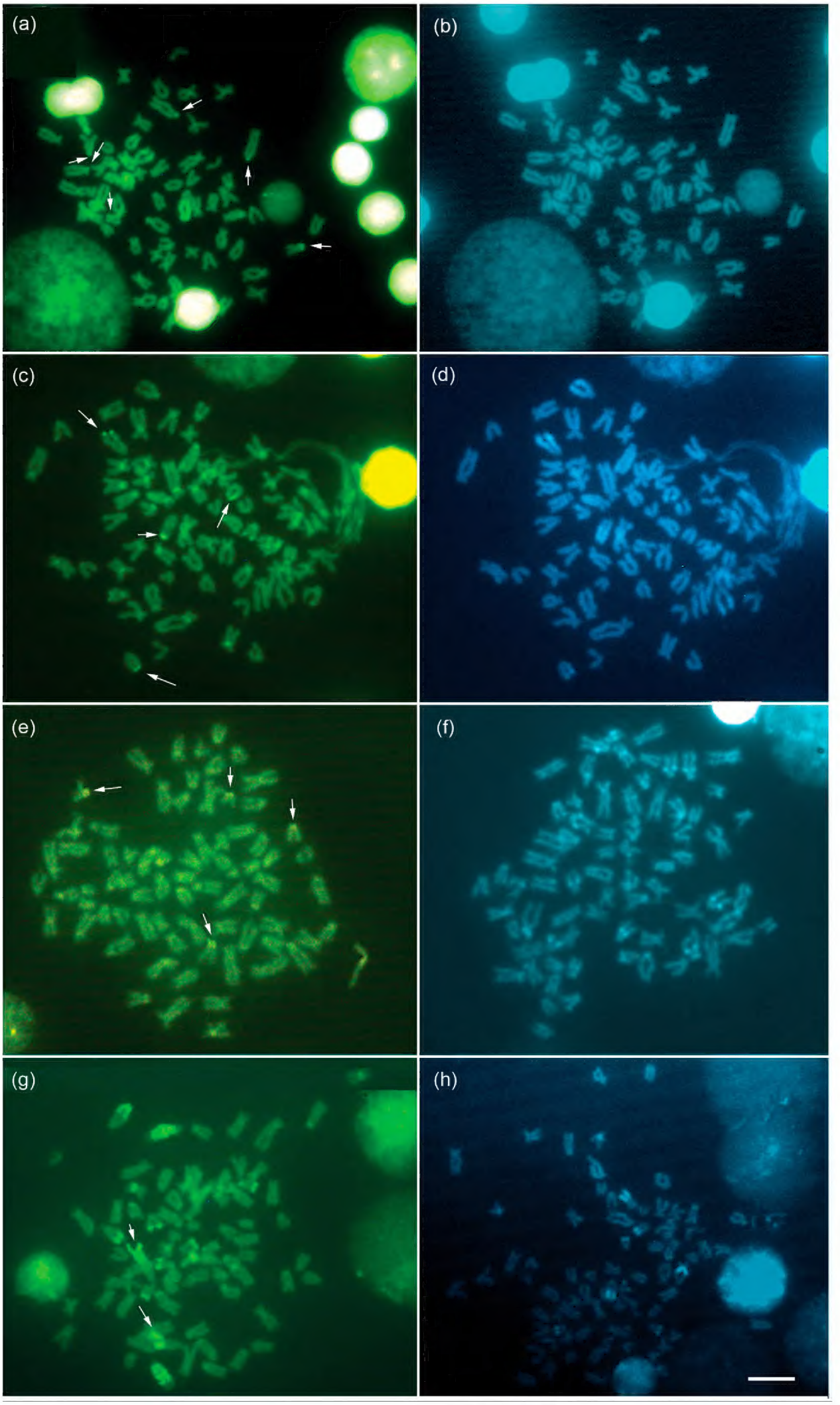


**Figure 2.** Metaphases stained with $CMA_3$ (left) and DAPI (right), of *H. ancistroides* **a, b** *H. strigaticeps* **c, d** *H. regani* **e, f** *H. paulinus* **g, h**. The arrows indicate the NOR-bearing chromosomes. Bar = 10 μm.

subtelocentric/acrocentric chromosomes, one of which was the NOR-bearing pair. In this pair, a heterochromatin block was located at the proximal portion of the secondary constriction, whereas three heterochromatin blocks, which occupied almost the entire long arm, were observed in a pair of subtelocentric/acrocentric chromosomes (pair 12) (Fig. 3d).

## Discussion

All species differed with respect to their diploid chromosome number and/or karyotype, as follows: 2n=68 (10m+26sm+32st-a) in *H. ancistroides* (Fig. 1a), 2n=72 (10m+16sm+46st-a) in *H. strigaticeps* (Fig. 1b), 2n=72 (10m+18sm+44st-a) in *H. regani* (Fig. 1c), and 2n=76 (6m+16sm+54st-a) in *H. paulinus* (Fig. 1d). This variability is consistent with the chromosomal data previously reported in the genus *Hypostomus*, which showed a wide variation in 2n (from 52 to 84) (Table 1). The available cytogenetic studies showed that the species that possess the same 2n have different karyotypes. In the same way as the features observed in *H. ancistroides* (2n=68) but with different fundamental numbers (FN) among different populations, i.e. 106, 102 and 96 (Michele et al. 1977, Artoni and Bertollo 1996, Alves et al. 2006) and the characteristics found in *H. regani*, the cytogenetic analysis showed the same diploid number (2n=72) and a FN of 102 and 116 (Artoni and Bertollo 1996, Alves et al. 2006, Mendes Neto 2008), also differing from those analyzed herein (Table 1). On the other hand, studies conducted by Michele et al. (1977) in *H. paulinus* and *H. strigaticeps* showed differences in both 2n and FN. This difference may be ascribed to the existence of different cytotypes in these species, the occurrence of cryptic species, problems with the species identification or with chromosomal classification.

According to Artoni and Bertollo (2001), 2n=54 is considered as a basal condition for the family Loricariidae. In a phylogenetic study of Loricariidae using morphological data, the genus *Hypostomus* was considered the most derived (Armbruster 2004), representing a group with more derived karyotypic forms, consisting mostly of st-a chromosomes with a high diploid number. It seems that there was a divergent karyotypic evolution among the *Hypostomus* species; on the other hand, two main chromosome rearrangements appear in the evolution of the genus: i) an increase in the diploid number (2n) in several species, probably due to centric fissions and ii) the same 2n but with a difference in the karyotype formula, probably accounted by pericentric inversions.

The same variability found in 2n and in karyotypes was also detected in NORs. Our data showed different phenotypes among the *Hypostomus* species, observed after silver staining and FISH. All species showed Ag-NORs and 18S rDNA sites located in the terminal regions of st-a chromosomes, but with a significant variation in number and location among them. *H. ancistroides* showed up to 8 NOR sites, all located on the short arms (Fig. 1a left and right boxes, respectively). *H. strigaticeps* showed NORs on the long arms and *H. regani*, NORs located on the short arms, and both species with up to 4 sites (Fig. 1b and 1c left and right boxes, respectively), and *H. paulinus* evidenced

only two NOR-bearing chromosomes located on the long arm (Fig. 1d left and right boxes), which could be considered as species-specific characteristics.

The presence of one pair of NOR-bearing chromosomes, and also its interstitial location seems to be a widespread condition for Loricariidae fish, since this occurs among the Neoplecostominae and Hypoptopomatinae species (Alves 2000). However, in Hypostomini, the occurrence of multiple NORs and their location in the terminal position is most common, as observed here and recorded by other authors (Artoni and Bertollo 1996, Kavalco et al. 2005, Alves et al. 2006). But the exact location and number of ribosomal sites are confirmed only by the FISH technique. With regard to the genus *Hypostomus*, the available molecular cytogenetic data on the location of ribosomal genes are few and restricted to 18S rDNA sites of *H. affinis* (Kavalco et al. 2005). These data are very important to prompt more discussions about the evolution of ribosomal DNA in this group.

In the four species presently studied, the NORs were positive for $CMA_3$ staining (Fig. 2), a feature that has been conserved among all Neoteleostei (Ráb et al. 1999). In addition, some other chromosomal regions were also considered GC-rich in the four species, mainly in *H. ancistroides* (Fig. 2a) and *H. paulinus* (Fig. 2g). *H. strigaticeps*, *H. regani*, and *H. paulinus* (Fig. 2d, f, h respectively) are three species that also showed several positive markers for DAPI staining, indicating AT-rich regions that were not found in *H. ancistroides* (Fig. 2b).

Some other studies carried out in *Hypostomus* (Artoni and Bertollo 1999, Kavalco et al. 2004, Cereali et al. 2008) also showed that this fish group may possess different classes of GC and/or AT-rich repetitive DNA families, as observed in the species analyzed in the present report. AT-rich regions are also rare among fishes, and have been reported mainly in some Hypostomini species (Artoni and Bertollo 1999, Kavalco et al. 2004, Rubert et al. 2008), some zebrafish species (Gornung et al. 1997, Phillips and Reed 2000), and gobiid fishes (Canapa et al. 2002).

The chromosome banding performed in all species analyzed showed a variation in the heterochromatin distribution pattern. However, the presence of heterochromatin in some chromosomes was constant, as observed in the pericentromeric region of a metacentric pair in *H. ancistroides* (pair 2), *H. strigaticeps* (pair 3), and *H. paulinus* (pair 1) (Fig. 3a, b, d, respectively), also reported in *H. nigromaculatus* by Rubert et al. (2008). An additional characteristic is the presence of some conspicuous blocks in the terminal regions of some st-a chromosomes of the karyotype. The same banding profile, organized in blocks, was also observed by others researchers: in *Hypostomus* sp. B from the Mogi Guaçu River (Artoni and Bertollo 1999), *H. affinis* (Kavalco et al. 2004), *H. cochliodon* (Cereali et al. 2008), and *H. nigromaculatus* (Rubert et al. 2008). Interestingly, in *H. paulinus*, pair 12 proved to be well differentiated, with the long arm almost entirely heterochromatic, a feature observed only in this species. On the other hand, *H. regani* showed a more distinct heterochromatin distribution in relation to the other species, with a preferential location in the interstitial regions of st-a chromosomes (Fig. 3c).

The presence of a marker chromosome that seems conserved for most *Hypostomus* species, corresponding to the NOR-bearing chromosome pair, which shows a heterochromatin block adjacent to this site (e.g. Artoni and Bertollo 1999, Kavalco et al.

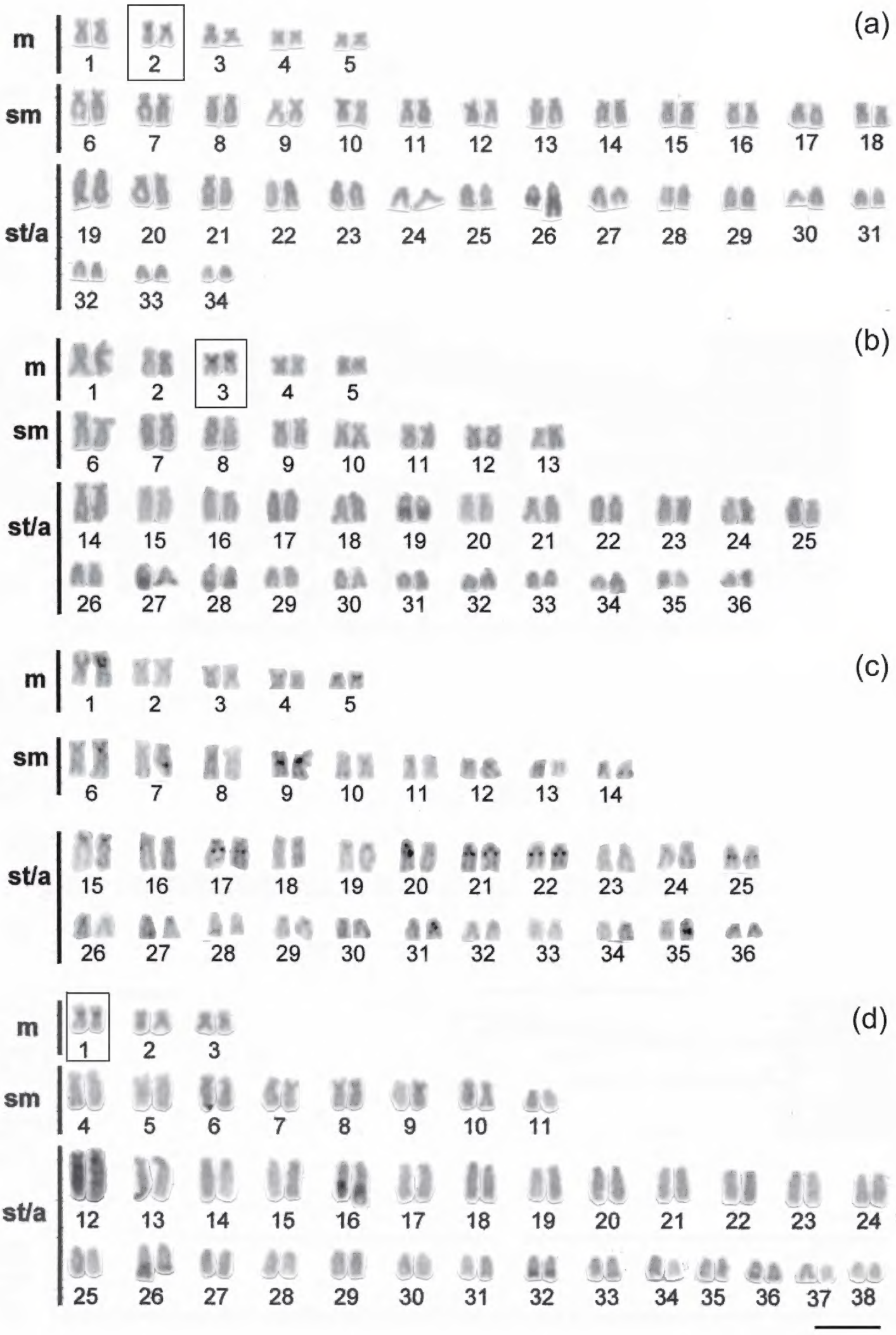


**Figure 3.** Karyotypes of **a** *H. ancistroides* **b** *H. strigaticeps* **c** *H. regani* and **d** *H. paulinus*, arranged from C- banded chromosomes Bar = 10 μm.

2004, Rubert et al. 2008), was also observed. It can be inferred from all data on the heterochromatin composition and distribution that each species has its own peculiarities, i.e., each species has a unique banding pattern.

Karyotypes, banding patterns, number and location of ribosomal DNA sites, and repetitive DNA are important tools for the cytotaxonomy of *Hypostomus* species. Since these characteristics do not vary among the different populations of the same species, they are significant cytogenetic markers at the species level.

Further data on other *Hypostomus* species from different rivers, as well as detailed studies of satellite DNA sequences may clarify important issues of genome organization, be used as genetic markers, and provide interesting insights for the comprehension of the evolution of this genus.

## Acknowledgments

The authors are grateful to Dr. Ana Lúcia Dias and Dr. André L. L. Vanzela for the review of this manuscript. This research was supported by a grant from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

## References

Alves AL (2000) Análise da evolução dos gêneros da subfamilia Hemipsilichthiinae (Ostariophysi, Siluriformes, Loricariidae) com base em caracteres cromossômicos e de DNA mitocondrial. Ph.D. Dissertation, Botucatu, Brazil: Instituto de Biociências, Universidade Estadual Paulista.

Alves AL, Oliveira C, Nirchio M, Granado A, Foresti F (2006) Karyotypic relationships among the tribes of Hypostominae (Siluriformes: Loricariidae) with description of XO sex chromosome system in a Neotropical fish species. Genetica 128: 1–9. doi: 10.1007/s10709-005-0715-1

Armbruster JW (2004) Phylogenetic relationships of suckermouth armoured catfishes (Loricariidae) with emphasis on the Hypostominae and Ancistrinae. Zoological Journal of the Linnean Society 141: 1–80.

Artoni RF, Bertollo LAC (1996) Cytogenetic studies on Hypostominae (Pisces, Siluriformes, Loricariidae). Considerations on karyotype evolution in the genus *Hypostomus*. Caryologia 49: 81–90.

Artoni RF, Bertollo LAC (1999) Nature and distribution of constitutive heterochromatin in fishes, genus *Hypostomus* (Loricariidae). Genetica 106: 209-214. doi: 10.1023/A:1003957719178

Artoni RF, Bertollo LAC (2001) Trends in the karyotype evolution of Loricariidae fish (Siluriformes). Hereditas 134: 201-210. doi: 10.1111/j.1601-5223.2001.00201

Artoni RF, Venere PC, Bertollo LAC (1998) A heteromorphic ZZ/ZW sex chromosome system in fish, genus *Hypostomus* (Loricariidae). Cytologia 63: 421–425.

Bertollo LAC, Takahashi CS, Moreira-Filho O (1978) Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). Brazilian Journal of Genetics 1: 103–120.

Camilo FM (2004) Estudos citogenéticos em algumas espécies de peixes da família Loricariidae pertencentes à bacia do rio Piracicaba. Ph.D. Dissertation, São Carlos, Brazil: Departamento de Genética e Evolução, Universidade Federal de São Carlos.

Canapa A, Cerioni PN, Barucca M, Olmo E, Caputo V (2002) A centromeric satellite DNA may be involved in heterochromatin compactness in gobiid fishes. Chromosome Research 10: 297–304.

Cereali SS (2006) Estudos citogenéticos de Loricariidae (Siluriformes) do Planalto da Bodoquena – Mato Grosso do Sul. Ph.D. Dissertation, Londrina, Brazil: Departamento de Biologia Geral, Universidade Estadual de Londrina.

Cereali SS, Pomini E, Rosa R, Zawadzki CH, Froehlich O, Giuliano-Caetano L (2008) Karyotype description of two species of *Hypostomus* (Siluriformes, Loricariidae) of the Planalto da Bodoquena, Brazil. Genetics and Molecular Research 7: 583–591.

Fenerich PC, Foresti F, Oliveira C (2004) Nuclear DNA content in 20 species of Siluriformes (Teleostei, Ostaryophysi) from the Neotropical region. Genetics and Molecular Biology 27 (3): 350-354. doi: 10.1590/S1415-47572004000300008

Gornung E, Gabrielli I, Cataudella S, Sola L (1997) $CMA_3$-banding pattern and fluorescence *in situ* hybridization with 18S rRNA genes in zebrafish chromosomes. Chromosome Research 5: 40–46.

Hatanaka T, Galetti Jr, PM (2004) Mapping of 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae). Genetica 122: 239–244. doi: 10.1007/s10709-004-2039-y

Howell WM, Black DA (1980) Controled silver staining of nucleous organizer regions with a protective colloidal developer: a 1-step method. Experientia 36: 1014–1015.

Kavalco KF, Pazza R, Bertollo LAC, Moreira-Filho O (2004) Heterochromatin characterization of four fish species of the family Loricariidae (Siluriformes). Hereditas 141: 237–242. doi: 10.1111/j.1601-5223.2004.01850.x

Kavalco KF, Pazza R, Bertollo LAC, Moreira-Filho O (2005) Karyotypic diversity and evolution of Loricariidae (Pisces, Siluriformes). Heredity 94: 180-186. doi: 10.1038/sj.hdy.6800595

Mendes Neto EO (2008) Estudos citogenéticos em algumas espécies de Loricariidae (Teleostei, Siluriformes) da região de transposição do rio Piumhi para o rio São Francisco. Ph.D. Dissertation, São Carlos, Brazil: Departamento de Genética e Evolução, Universidade Federal de São Carlos.

Michele JL, Takahashi CS, Ferrari I (1977) Karyotypic studies of some species of the family Loricariidae (Pisces). Cytologia 42: 539–546.

Montoya-Burgos JI, Muller S, Weber C, Pawlowski J (1998) Phylogenetic relationships of the Loricariidae (Siluriformes) based on mitochondrial rRNA gene sequences. In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS (Eds) Phylogeny and Classification of Neotropical Fishes. Porto Alegre, 363–374.

Muramoto J, Ohno S, Atkin NB (1968) On the diploid state of the fish order Ostariophysi. Chromosoma 24: 59–66.

Phillips RB, Reed KM (2000) Localization of repetitive DNAs to zebrafish (*Danio rerio*) chromosomes by fluorescence in situ hybridization (FISH). Chromosome Research 8: 27–35.

Ráb P, Rábová M, Reed KM, Phillips RB (1999) Chromosomal characteristics of ribosomal DNA in the primitive semionotiform fish, longnose gar *Lepisosteus osseus*. Chromosome Research 7: 475–480.

Reis RE, Pereira EHL, Armbruster JW (2006) Delturinae, a new loricariid catfish subfamily (Teleostei, Siluriformes), with revisions of *Delturus* and *Hemipsilichthys*. Zoological Journal of the Linnean Society 147: 277–299.

Rubert M, Zawadzki CH, Giuliano-Caetano L (2008) Cytogenetic characterization of *Hypostomus nigromaculatus* (Siluriformes: Loricariidae). Neotropical Ichtyhology 6: 93–100. doi: 10.1590/S1679-62252008000100011

Schweizer D (1980) Simultaneous fluorescent staining of R bands and specific heterochromatic regions (DA-DAPI bands) in human chromosomes. Cytogenetics and Cell Genetics 27: 190-193. doi: 10.1159/000131482

Sumner AT (1972) A simple techinique for demonstrating centromeric heterochromatin. Experimental Cell Research 75: 304-306. doi: 10.1016/0014-4827(72)90558-7

Swarça AC, Giuliano-Caetano L, Vanzela ALL, Dias AL (2001) Heteromorphism of rDNA size in *Pinirampus pirinampu* (Pisces: Pimelodidae) detected by in situ hybridization. Cytologia 66: 275–278.

Weber C (2003) Subfamily Hypostominae. In: Reis RE, Kullander SO, Ferraris Jr CJ (Eds) Check list of the freshwater fishes of South and Central America. Porto Alegre, 351–372.

Zawadzki CH, Renesto E, Reis RE, Moura MO, Mateus RP (2005) Allozyme relationships in hypostomines (Teleostei: Loricariidae) from the Itaipu Reservoir, Upper Rio Paraná basin, Brazil. Genetica 123: 271-283. doi: 10.1007/s10709-004-5418-5

Zawadzki CH, Weber C, Pavanelli CS (2008) Two new species of *Hypostomus* Lacépède (Teleostei: Loricariidae) from the upper rio Paraná basin, Central Brazil. Neotropical Ichtyhology 6: 403-412. doi: 10.1590/S1679-62252008000300013